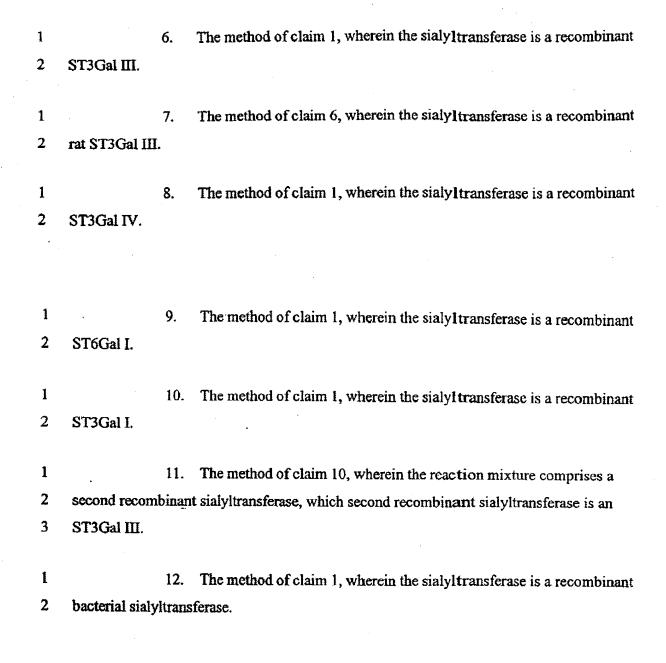
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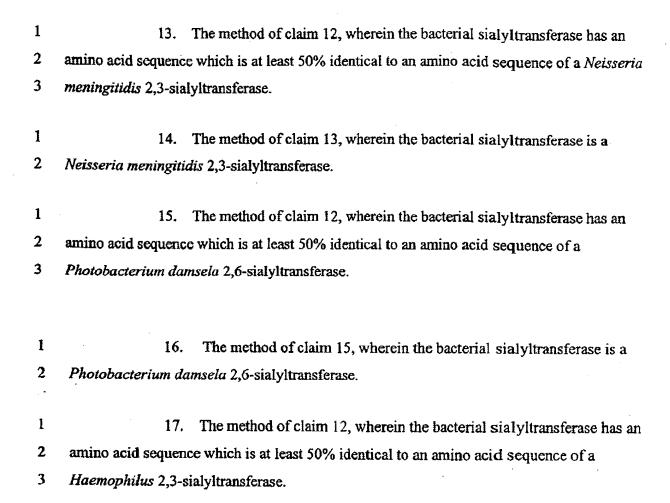
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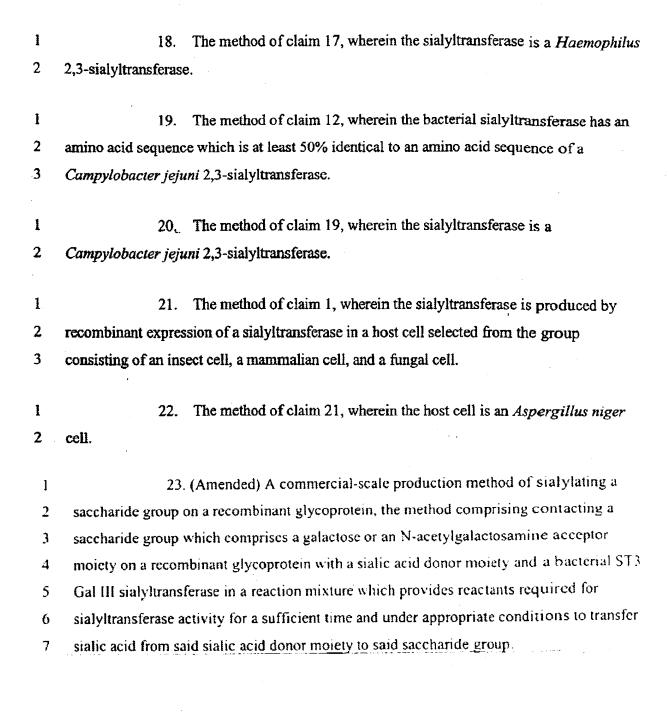
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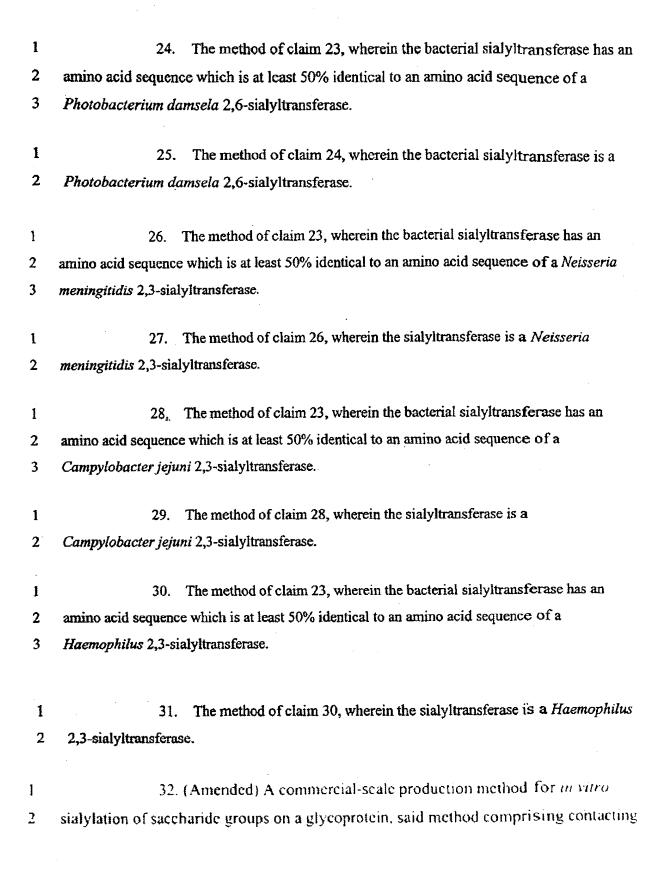
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- 1. (Amended) A commercial-scale production method of sialylating a 1 saccharide group on a recombinant glycoprotein, the method comprising contacting a 2 saccharide group which comprises a galactose or N-acetylgalactosamine acceptor moiety 3 on a recombinant glycoprotein with a sialic acid donor moiety and a recombinant ST3 4 Gal III sialyltransferase in a reaction mixture which provides reactants required for 5 sialyltransferase activity for a sufficient time and under appropriate conditions to transfer 6 sialic acid from said sialic acid donor moiety to said saccharide group. 7 2. The method of claim 1, wherein the sialic acid donor moiety is CMP-
- The method of claim 1, wherein the sialic acid donor moiety is CMPsialic acid.
- The method of claim 2, wherein the CMP-sialic acid is enzymatically generated in situ.
- 4. The method of claim 1, wherein the sialyltransferase is a recombinant eukaryotic sialyltransferase which substantially lacks a membrane-spanning domain.
- 1 5. The method of claim 1, wherein the sialyltransferase includes a sialyl
- 2 motif which has an amino acid sequence that is at least about 40% identical to a sialyl motif
- 3 from a sialyltransferase selected from the group consisting of ST3Gal I, ST6Gal I, and
- 4 ST3Gal III.









Application/Control Number: 10/081,455 Art Unit: 2611 said saccharide groups with a ST3 Gal III sialyltransferase, a sialic acid donor moiety, 3 and other reactants required for sialyltransferase activity for a sufficient time and under 4 appropriate conditions to transfer sialic acid from said sialic acid donor moiety to said 5 saccharide group. 6 1 The method of claim 32, wherein the sialyltransferase is present at a 33. 2 concentration of between about 5-25 mU per mg of glycoprotein. 1 34. The method of claim 32, wherein the sialyltransferase is present at a 2 concentration of between about 10-50 mU/ml of reaction mixture and the glycoprotein is 3 present in the reaction mixture at a concentration of at least about 2 mg/ml. 1 The method of claim 32, wherein the method yields a glycoprotein having sialylation of at least about 80% of terminal galactose residues present on the 2

The method of claim 32, wherein the sialyltransferase is a recombinant

3

1

2

saccharide groups.

sialyltransferase.

36.

